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Leukemic marker detection using a spectro-polarimetric surface plasmon resonance platform

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ABSTRACT

In this paper, we present a proof of concept screening for monoclonal immunoglobulin as a leukemia tumor marker using a surface plasmon resonance (SPR) bio-sensing platform. This screening method is based on measurements of immunoglobulin levels in human serum and the determination of the relative concentrations of kappa and lambda light chains. The kappa/lambda ratio is used to determine the presence of monoclonal immunoglobulin. Tests have been performed using standard solutions of immunoglobulins and serum samples from patients with known leukemic diagnoses. This platform has a resolution of 5×10^{-7} refractive index unit (RIU) per channel, which is up to 10 times better than other SPR imaging systems for multi-sensing applications. The results obtained with this technique are in agreement with those acquired using conventional methods for immunoglobulin detection, indicating that our polarimetric SPR platform should be suitable for a cheap and efficient tool for early leukemia biomarker screening and monitoring applications.

1. Introduction

Leukemic proliferation of B-cell tumors, including plasma cell neoplasms, Waldenström's disease, chronic lymphocytic leukemia (CLL) and lymphomas, is often associated with monoclonal immunoglobulin. In Canada, about 5800 people are diagnosed and 2600 die yearly of leukemic malignancies (Canadian Cancer Statistics, 2013). According to the American cancer society (American Cancer Society, 2013), there is no standard screening technique for early detection of leukemia which is generally diagnosed by morphological and phenotypic examinations when clinical symptoms appear. In addition, techniques currently used for leukemia diagnosis, such as blood cell morphology, flow cytometry or bone marrow biopsy, are not suitable for screening as they are expensive, need a long analysis time and require highly qualified professionals.

In the last two decades, important studies have been performed on serum components, such as immunoglobulin (Bradwell et al., 2013) and their light chain (Campbell et al., 2013; Katzmann et al., 2002; Rajkumar et al., 2005), in a range of plasma cell and B-cell neoplastic disorders in order to discover new biomarkers for leukemia diagnosis. In the specific case of CLL, a large proportion of articles in the literature suggests that a comparative analysis of the amounts of kappa and lambda light chains should provide a strong indication of the presence of the disease (Maurer et al., 2011; Pratt et al., 2009; Rajkumar et al., 2005).

Immunoglobulin classes and the different light chains, such as IgGκ, IgGλ, IgMk and IgMλ can be identified by specific antibodies. A skewed κ–λ ratio may be found in patients with asymptomatic monoclonal immunoglobulin. By using the serum protein electrophoresis method, a κ–λ ratio interval between 0.6 and 4.2 may predict significant monoclonal components in patients with clinical disease (Bergón et al., 2005). More recently, free light chains in serum are used as a sensitive biomarker in screening, monitoring and risk stratifying of patients with myeloma, CLL and B-cell lymphomas. Abnormal free light chain ratio has been shown as a prognostic factor for reduced survival of patients with CLL. The patients with an abnormal light chain ratio are more likely to have genetic changes associated with aggressive disease progression and require early treatment (Charafeddine et al., 2012).

The aim of this study is to propose and validate a cost-effective method for real-time determination of relative concentrations of different types of immunoglobulins in human serum for leukemia screening. Concentrations of these markers were monitored using surface-plasmon resonance (SPR) bio-sensing. SPR has become one of the dominant technologies for bio-detection, as it enables

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real-time and label-free detection of biological species without requiring a large amount of samples (low volume of analyte (Valsecchi and Brolo, 2013)). An SPR-based method also provides good sensitivity and low limit of detection (Homola, 2006).

The reported SPR biosensor was able to selectively detect light chain immunoglobulin from both test solutions and samples of human sera obtained from leukemia patients. To assure specific adsorption of the analytes, an existing technique of antibody immobilization has been transferred to gold surface (Johnson and Mutharasan, 2012; Saha et al., 2003).

Finally, this SPR-based biosensing platform should provide a cheap and efficient tool for early leukemia screening. This technology should also be useful for monitoring the progression of leukemia patients during therapy.

2. Principle and instrumentation

2.1. Principle

The principle of leukemic marker detection is summarized in Fig. 1. Mature B-cell leukemia, lymphoma, myeloma and other plasma cell disorders are clonal proliferation of immunoglobulin-producing cells. Monoclonal overexpression results in a dominant type of immunoglobulin that are composed of a specific heavy chain for IgG or IgM (rare occasions IgA, IgD or IgE) and restricted to either κ or λ light chain, as illustrated in Fig. 1. Therefore, the presence of monoclonal immunoglobulin can be detected by a skewed concentration ratio (relative to their usual distribution in the blood of healthy individuals) between the kappa and lambda light chains (Jain and Rai, 2011). This measurement of the κ–λ light chain ratio in human serum may then be used for leukemia screening, as well as a monitoring tool for the disease response during treatment. The κ–λ light chain ratio for non-leukemic patients has been determined to fall in a range from 1.4 to 2, whereas ratio for the leukemic patients is greater than 2 for κ overexpressed light chain and much lower than 1 for λ (Katzmann et al., 2002).

2.2. Instrumentation

A biosensor suitable for leukemia screening needs to fulfill several requirements. First, a wide dynamic range is necessary since the light chain concentrations in serum vary extensively from a patient to another. It ranges from 0.1 to 1 mg/mL, according to data acquired with conventional techniques (turbidimetry assays and serum protein electrophoresis) on samples used in this paper. Taking into account molecular size, surface chemistry, volume and surface concentration, the system is required to operate with a resolution of about 10^{-6} refractive index unit (RIU), and a dynamic range greater than 10^{-3} RIU. In order to get reliable and comparable data, biomarkers analysis must be performed with the exact same experimental condition. To answer this need, the system has to include a multi-sensing scheme to simultaneously measure the κ–λ light chain ratio in a sample of human blood. The polarimetric method using spectral polarization reading has been chosen as it provides good resolution and wide dynamic range. Fig. 2 shows a schematic of the apparatus. It is based on the measurement of p-polarized reflected light, affected by the SPR coupling (Raether, 1988), referenced to s-polarized light, which is not affected by SPR, as seen in the experimental curve in Fig. 2 inset.

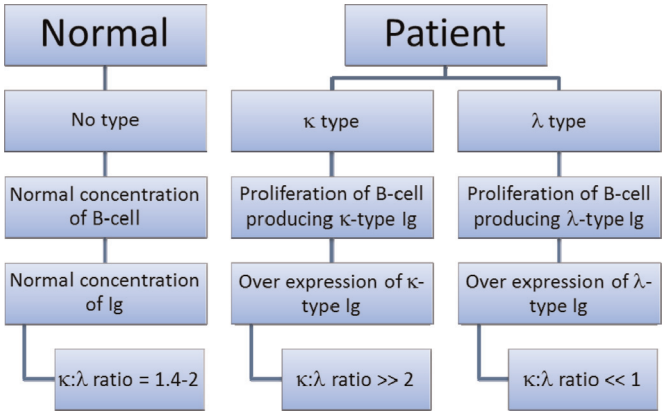

Fig. 1. Chart illustration of a normal (healthy) person and leukemic patients presenting over expression of either κ or λ light chains.

The ratio of these polarizations gives the tangent value of the polarization vector (Hecht, 2002). The spectral position of the resonance, defined by the minimum of the measured reflectivity curve (see Fig. 2), is tracked with a polynomial interpolation and roots calculation. The details of the optical setup for the SPR system used in these experiments are presented in Fig. 2a. A white-light beam incoming from a LED (Thorlabs warm white 450–750 nm – MWWHL3) was focused in a multimode fiber (Thorlabs MM core 105 μm – M15L01). Light, at the output of the fiber, was then brought to an achromatic lens (L1) and the beam was collimated before passing through a plate polarizer. This parallel beam was directed to the sensing block, which consists of a microscope plate covered with a 50 nm gold layer in contact with the tested solution on one side (two channels – the flow cell back view is also presented in Fig. 2a) and with oil immerse BK7 prism on the other side for coupling condition. Reflected light passed through an analyzer before going to the spectro-imaging system. A CCD camera (Andor Newton 971) acquired the light dispersed from the monochromator and the generated image was then processed to get useful spectral information from the two channels.

Finally, characterization tests have been realized on the system to determine its performance. The resolution was determined by using different solutions of ethanol in water with concentrations of 0.1, 0.2 and 0.5 vol% as shown in Fig. 2b. Using the refractive index changes of the ethanol solutions (Lide, 2005) and the measured signal-to-noise ratio, the setup resolution was calculated to be 5×10^{-7} RIU, which is better than the needed resolution for leukemia screening. In terms of dynamic range, the spectral window offered by the instrument is about 44 nm which is equivalent to a change of 1.5×10^{-3} RIU and thus respecting the needed specifications.

3. Materials and methods

3.1. Materials and samples

All the tests have been performed in 10 mM Phosphate Buffer Saline (pH 7.4 PBS 10 ×, Sigma-Aldrich P5493 diluted 10 times in 18 MΩ cm deionized water). Lyophilized recombinant Protein G (Sigma-Aldrich P4689) from *E. coli* (MW ~ 20 kDa) was used in all the experiments as well as Bovine Serum Albumin (BSA) from Sigma-Aldrich (A7906). Goat polyclonal anti-human kappa (Sigma-Aldrich K3502) and goat polyclonal anti-human lambda (Sigma-Aldrich L1645) were used respectively to bind to kappa and lambda light chains.

Patient serum samples were collected at the Mount-Sinaï Hospital, Toronto. The 4 serum samples in this study included a normal control and 3 samples from patients presenting an over-expression of a monoclonal type of immunoglobulin (G-kappa, M-lambda and G-lambda respectively). Total concentrations of